Intramolecular Nucleophilic Catalysis of Glucoside Hydrolysis by the 2-Phosphate Group

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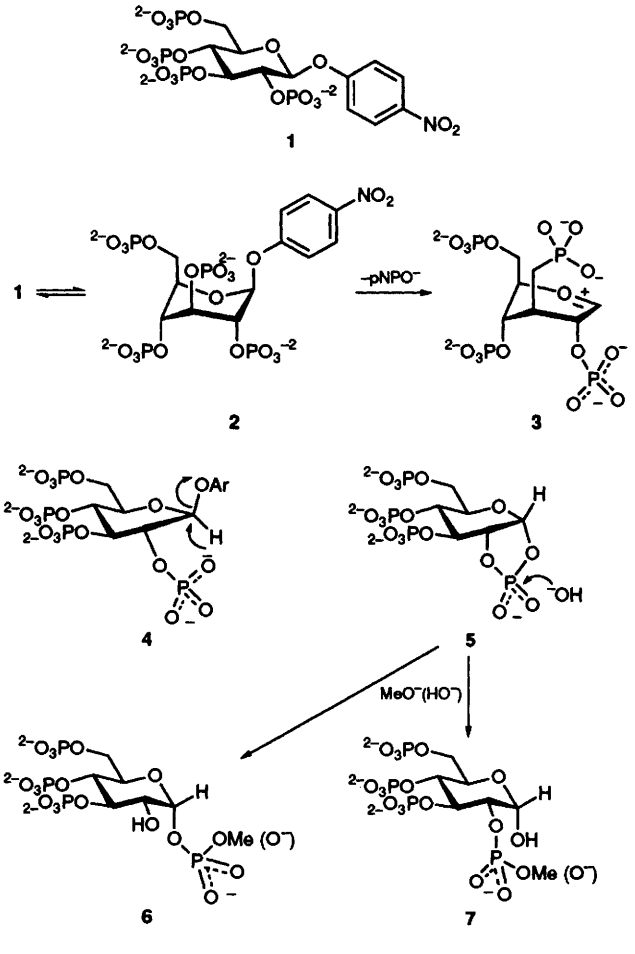
4-Nitrophenyl β-D-glucoside tetraphosphate is hydrolysed 100 times faster than the 2-methoxy derivative, by way of the 1,2-cyclic phosphate diester, which is solvolysed by attack at phosphorus.

Retaining glycosidases[1] such as lysozyme achieve rapid cleavage of the glycosidic bond by combining efficient general acid catalysis of the departure of the leaving group[2] with assistance from a second, typically CO_2^-, group. This could act in various ways, of which nucleophilic catalysis seems chemically most likely, though direct evidence is lacking.[3] We report that a neighbouring 2-phosphate dianion can act as an effective nucleophilic catalyst in the hydrolysis of the model glucoside **1**. This compound was of interest because of a report[4] that *myo*-inositol hexaphosphate, which has one axial and five equatorial phosphate groups, is converted at high pH to the inverted chair structure, which would have five axial phosphate groups, all presumably in the dianion form. We wondered whether the same process might occur to a significant extent with a glycoside tetraphosphate (the extreme case is represented by **1** ⇌ **2**). If this happens a loose electrostatic sandwich structure **3**, of the sort discussed[3] for a possible lysozyme transition state would be generated, and the hydrolysis of the glucoside should be accelerated.

The pH–rate profile for the hydrolysis of **1** is compared in Fig. 1 with data for two 'parent' compounds. The obvious comparison, with 4-nitrophenyl β-D-glucoside itself, is not immediately helpful because the hydrolysis of this compound is base catalysed above pH 7, probably by way of the 2-O$^-$ form.† So we prepared the 2-OMe derivative of 4-nitrophenyl β-D-glucoside. (For this compound base-catalysed hydrolysis appears above pH 10: the reaction involves nucleophilic aromatic substitution by hydroxide ion, as shown most conveniently by its rate, which is almost identical to that for the reaction of 4-nitroanisole under the same conditions.)

The reactions of interest are those occurring in the pH-independent regions, above pH 9 for (the octa-anion of) **1**, below pH 9 for 2-O-methyl 4-nitrophenyl β-D-glucoside, and below pH 6 for the parent 4-nitrophenyl β-D-glucoside. The spontaneous hydrolysis of the tetraphosphate octa-anion is 100 times faster than that of the parent compound, and 70 times faster than that of the 2-methoxy derivative (Fig. 1).

Possible explanations are a degree of electrostatic stabilisation of the transition state as suggested by structure **2**, and nucleophilic catalysis by the 2-phosphate group. The two are not mutually exclusive: nucleophilic catalysis (**1** → **5**) will also be favoured, stereoelectronically as well as electrostatically, in a compromise conformation such as **4**: while **3** (even if it exists as drawn, which is unlikely in view of the evidence[6] that



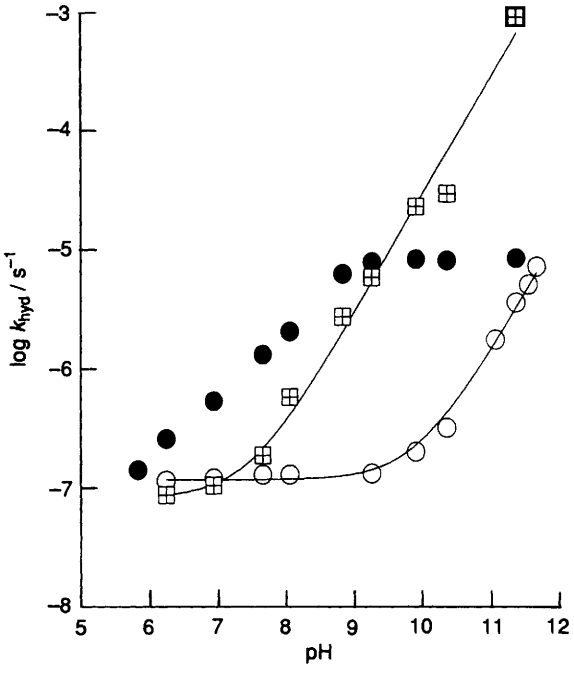


Fig. 1 pH–rate profiles for the hydrolysis of 4-nitrophenyl β-D-glucoside tetraphosphate (**1**, filled circles) compared with data for the hydrolysis of the parent 4-nitrophenyl β-D-glucoside (squares) and its 2-*O*-methyl derivative (open circles). Measurements were at 80 °C and ionic strength one mol dm^{-3} (KCl). The curves drawn for the two parent compounds are calculated, using (least squares) values of k_0 and k_{OH} of 8.28 × 10^{-8} and 1.16 × 10^{-7} s^{-1}, and 1.19 × 10^{-2} and 5.04 × 10^{-5} dm^3 mol^{-1} s^{-1} for the 2-OH and 2-OMe compounds, respectively. The points are experimental.

glucosyl cations are too unstable to be full intermediates in water in the presence of nucleophilic anions) would collapse to the same cyclic diester **5**.

5 is clearly an intermediate. It does not accumulate under the basic conditions, but when the reaction is run in 50 mol% aqueous methanol (0.1 mol dm^{-3} in NaOH) a major product (some 50% by ^1H NMR in D_2O) has a methyl doublet (δ 3.5, J 10.3 ± 0.4 Hz:), as expected for a methyl phosphate. The ^{31}P spectrum (in D_2O containing 0.1 mol dm^{-3} NaOD) shows (apart from inorganic phosphate) a product with a quartet at δ 4.8, with 10.1 ± 0.4 Hz coupling to three equivalent protons, identified (by the addition of an authentic sample to the NMR tube) as methyl phosphate itself. The only strong, resolved anomeric proton appears as a double doublet at δ 5.47 ($^3J_{HH}$ 3.5, and $^3J_{HP}$ 7.0 Hz), closely similar to the anomeric proton signal of β-D-glucose pentaphosphate.[5] We interpret these observations in terms of competing attack by hydroxide and methoxide ions at the reactive phosphorus centre of **5**,‡ with displacement of either oxygen of the cyclic diester.§

Of the four expected products, [the α-D-1-phosphate monoester and methyl phosphate diester **6**(OH) and **6**(OMe), and the 2-phosphate monoester and methyl phosphate diester **7**(OH) and **7**(OMe)], only the phosphate monoesters are likely to be stable under the vigorous conditions (0.1 mol dm^{-3} NaOH, 80 °C). the 1- and 2-diesters will both lose (methanol or) methyl phosphate, a better leaving group than 4-nitrophenoxide, by different mechanisms. The key observation is the phosphorylation of methanol: this can only reasonably be explained by the formation of the 1,2-cyclic phosphate diester, and is thus direct evidence for intramolecular nucleophilic catalysis by the phosphate group.¶

We thank the Swedish Natural Science Research Council for support.

Received, 23rd December 1993; Com. 4/00094C

Footnotes

† The reaction of the α-4-nitrophenyl glucoside tetraphosphate is pH-independent from pH 7.6–12, and 23 times slower than that of **1** above pH 9: but could involve direct attack of the 2-phosphate group on the adjacent aromatic ring.[5]

‡ It is clear that attack at phosphorus is the major pathway. We see no evidence for the formation of any methyl glucoside.

§ *Note added in proof:* We have now observed **5** directly, in an experiment carried out at pH 8.82 (borate buffer), as a signal in the ^{31}P NMR at δ 15.54, which appears and subsequently disappears at rates consistent with the rates observed for the overall reaction.

¶ Convincing but indirect evidence for intramolecular nucleophilic catalysis by the amide group of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was presented by D. Piszkiewicz and T. C. Bruice, *J. Am. Chem. Soc.*, 1967, **89**, 6237.

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